Available online at www.sciencedirect.com
ScienceDirect
Industrial Crops and Products 26 (2007) 100–104
INDUSTRIAL CROPS AND PRODUCTS
AN INTERNATIONAL JOURNAL
www.elsevier.com/locate/indcrop

Short communication

Grain-protective properties of herbal extracts against the bean weevil *Acanthoscelides obtectus* Say

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Received 30 June 2006; received in revised form 24 January 2007; accepted 31 January 2007

Abstract

Studies were conducted to evaluate the effect of the ethanol extracts from five aromatic medicinal plants against the bean weevil, *Acanthoscelides obtectus* (Coleoptera: Curculionidae). The extracts were tested for potential to protect stored legume seeds in terms of their repellency, toxicity and reduction of F1 progeny. Significant insecticidal activity was exhibited only by the 100% concentrated extracts from *Urtica dioica* L. and *Taraxacum officinale* L., whereas both 100 and 30% extracts from these plants were effective in repellency and reduction of F1 progeny. Although the extract form *Achillea millefolium* L. (100%) was ineffective in insecticidal activity, it provided a good level of repellency and reduction of F1 progeny. Extracts from *Sambucus nigra* L. and *Juglans regia* L. were ineffective in all conducted bioassays.

Keywords: Medicinal plants; Insecticidal and repellent bioassay; Botanical insect control agent

1. Introduction

Although the problems concerning the pest resistance and healthy environment are in the focus of public interest, the protection of stored grain using the conventional insecticides is still the prevailing method of insect control (Subramanyam and Hagstrum, 1995). Therefore, there is an interest in finding alternative ways for stored products protection, especially within the scope of organic and natural products that are popularly used for same purpose in the ethno-tradition. The present studies were undertaken to evaluate the effectiveness of different plant extracts to protect bean seeds against the bean weevil *Acanthoscelides obtectus*. The bean weevil is considered as one of the most serious stored bean pests worldwide. All extracts were obtained from the plants that are well known as non-noxious and widely used both in human nutrition and traditional medicine (Tucakov, 1973). The insecticidal properties of chosen plants were indicated by their local use to repel or kill pests and based on their major constituents known to be biologically active against insects, e.g. sesquiterpene lactones (*Achillea millefolium* and *Taraxacum officinale*) (Kovačević, 1995), juglone from leaves of *Juglans*

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doi:10.1016/j.indcrop.2007.01.010

regia (Sarić, 1989) and formic acid and sambunigrin from *Urtica dioica* and *Sambucus nigra*, respectively (Sekulović et al., 1996).

2. Material and methods

2.1. Preparation of the extracts

The plant material from five plants was used: below-ground parts from *T. officinale*, flowers from *S. nigra*, leaves from *U. dioica*, leaves from *J. regia* and flowers from *A. millefolium*, all collected during May, June and July 2004 at Maljen Mountain, Serbia. The identification of plants was confirmed by Z. Popović, Institute for Biological Research, Department of Ecology, Belgrade. To obtain the extracts from dried plant material, a method of two stage percolation was used (Federal Bureau of Health Care, 1984). Extraction was conducted with 70 vol% ethanol during 48 h. The ratio of initial dried plant material and extract yield was 1:1 (the yield of obtained solution was equal to initial dry weight of plant material). These extracts (called 'primary' extracts) were denoted as 100% concentrated. By diluting the 'primary' extracts, the 30% concentrated extracts were obtained (i.e. 30 g of 100% extract was diluted with 70 g of 70 vol% ethanol).

2.2. Test insect

The adult insects *A. obtectus* adults were obtained from stock cultures at the Institute for Biological Research, Belgrade, and were maintained on a common bean without exposure to any insecticide, in a controlled temperature and humidity chamber at $27 \pm 1\,^{\circ}C$, 65 ± 5 r.h. and L16:D8.

2.3. Screening on contact toxicity of extracts

Although the adults of *A. obtectus* do not feed on beans, the screening on toxicity of extracts both on glass and on substrate was conducted to clarify if the toxicity was achieved through the contact effect. One experiment was settled at glass medium: the aliquot of 0.3 ml of each tested solution was placed in petri dish (9 cm diameter), and the same aliquot of alcohol 70 vol% was used as control. Each treatment was replicated four times. The second experiment was settled at substrate, i.e. treated bean: a quantity of 100 g of bean per treatment was mixed with test solutions (3 ml of solution with 100 g of bean) in 500-ml glass and stirred 10 min with a rotary shaker (Multifix GmbH, Germany). Each treatment was replicated four times. In both experiments, the ethanol was allowed to evaporate for 20 min prior to the introduction of 20 unsexed adults (1–2 days old) of insect separately into each dish and these were kept at $27 \pm 1\,^{\circ}C$, 65 ± 5 r.h. and L16:D8.

Dead insects in petri dishes were counted at 4, 12, 24 and 48 h. The insects were considered as dead if appendages did not move when prodded with a fine brush. Dead insects from substrate were removed after the sieving through the sieve with 5 mm diameter holes. After the prodding with a brush, a few minutes was spent to assure if the individuals were dead or just paralyzed. If they moved, they were introduced again on substrate.

Percentage mortality was calculated using Abbott's (1925) correction formula for natural mortality in untreated controls. Insecticidal effect of extracts both on glass and treated beans was not significantly different, which approved that further investigation on toxicity should not be attributed to the ingestion of treated bean (Popović et al., 2006).

2.4. Insecticidal activity of extracts

For evaluation the insecticidal effect of the extracts on adult mortality of *A. obtectus*, the beans were treated separately with solutions of plant extracts. An aliquot of 3 ml of each test solution (extracts from five plants in both 30 and 100% concentrations) was mixed with 100 g of bean in 500-ml glass jars and stirred continuously for 10 min with a rotary shaker (Multifix GmbH, Germany) to ensure even spread of the materials over the surface of the beans. Samples were kept for 20 min to allow the solvent to evaporate completely. The beans were placed in plastic containers (9 cm high and 7 cm in diameter). Twenty unsexed 1–2 days old adults of *A. obtectus* were placed on the beans. The containers were covered with the fixed cotton cloth. Experiment was conducted at $27 \pm 1\,^{\circ}C$, 65 ± 5 r.h. and L16:D8, with each treatment repeated four times. The number of dead insects in each container was counted during the life cycle of adult (at 24, 48 and 96 h, and after 1, 2 and 3 weeks). The insects were sieved through the sieve with 5 mm diameter holes. Sieved individuals were considered as dead if appendages did not move when prodded with a fine brush immediately after sieving, and again after 5 min. Percentage mortality was calculated using the corrected formula of Abbott (1925).

2.5. Repellency bioassay

For evaluation of repellent effect of applied extracts we used the cup bioassay technique (Kumar et al., 2004). A quantity of 100 g of bean was treated with the 3 ml

Table 1
Mortality of *A. obtectus* exposed to beans treated with plant extracts

Plant extract	Percent mortality of insects					
	24 h	48 h	96 h	1 week	2 weeks	3 weeks
Control (70 vol% eth.)	0 f	0 e	0 f	0 f	11.0 ± 0.9 e	85.0 ± 1.2 c
U. dioica (Lf) (30%)	8.0 ± 2.1 c	12.4 ± 2.4 c	13.1 ± 0.9 c	14.1 ± 0.8 d	14.9 ± 1.1 d	91.0 ± 1.0 b
U. dioica (Lf) (100%)	22.9 ± 2.3 b	40.9 ± 2.9 b	47.8 ± 1.0 b	48.5 ± 1.8 b	55.8 ± 2.8 b	100.0 ± 0.0 a
T. officinale (Bp) (30%)	4.1 ± 0.4 e	4.1 ± 0.3 d	14.2 ± 0.6 c	14.4 ± 0.0 d	21.7 ± 0.5 c	95.6 ± 0.5 b
T. officinale (Bp) (100%)	48.9 ± 2.5 a	60.6 ± 2.5 a	65.4 ± 0.8 a	68.2 ± 2.5 a	82.9 ± 2.8 a	100.0 ± 0.0 a
A. millefolium (Fl) (30%)	5.1 ± 0.5 e	5.5 ± 0.5 d	6.0 ±0.4 d	16.6 ± c	23.4 ± 0.3 c	86.1 ± 0.1 c
A. millefolium (Fl) (100%)	5.1 ± 0.5 e	6.1 ± 0.4 d	6.3 ± 0.3 d	17.1 ± 0.4 c	17.4 ± 0.2 d	87.8 ± 0.2 c
S. nigra (Fl) (30%)	0 f	0 e	0 f	0 f	12.0 ± 0.8 e	84.8 ± 0.0 c
S. nigra (Fl) (100%)	6.2 ± 0.6 d	12.5 ± 2.5 c	12.5 ± 0.6 c	12.7 ± 0.8 d	13.4 ± 0.8 e	87.1 ± 1.0 c
J. regia (Lf) (30%)	4.3 ± 0.0 e	5.1 ± 0.6 d	5.3 ± 0.3 e	5.8 ± 0.0 e	16.0 ± 0.5 d	86.4 ± 0.7 c
J. regia (Lf) (100%)	4.1 ± 0.0 e	4.4 ± 0.3 d	4.7 ± 0.2 e	6.0 ± 0.7 e	16.0 ± 0.5 d	90.2 ± 0.7 b

Values are means of four observations (± S.E.). In a column, means followed by the same letter are not significantly different by Duncan's multiple range test ($P = 0.05$). Lf: extract from leaves; Bp: extract from belowground plant parts; Fl: extract from flowers.

of each plant extract at concentrations of 30 and 100% on a w/w basis. The treated bean was placed in a covered plastic container (8 cm × 8 cm × 10 cm) which was perforated on lateral sides with holes large enough to allow weevils to pass through (perforations were about 5 mm in diameter). Twenty 1–2 days old unsexed individuals of *A. obtectus* were placed on the bean in the center of the perforated container. Each of these containers with insects was placed in larger plastic container (14 cm × 14 cm × 15 cm), which was covered with fixed cotton cloth. Experiment was conducted at 27 ± 1 °C, 65 ± 5 r.h. and L16:D8, with each treatment repeated four times. The repellency of the plant extracts was measured in terms of the percentage of insects moving out of the container, away from the treated bean (Mohan and Fields, 2002). All escaped insects which were present in the larger container at 4, 24 and 48 h after the introduction of insects were counted and removed at every observation time.

2.6. Effectiveness of extracts on reduction of number of individuals in F1 progeny

To avoid the undesirable handling effects due to sexing the insects under the microscope, unsexed adults were introduced to treated beans in relatively high number per plot (Koona and Dorn, 2005). Twenty unsexed, 1–2 days old adult insects were randomly collected from the stock jar and introduced into plastic container (9 cm high and 7 cm in diameter) with beans treated (100 g of beans has been stirred for 10 min with 3 ml of test solution and allowed to evaporate for 20 min). The containers were covered with a fixed cotton cloth and kept at 27 ± 1 °C, 65 ± 5 r.h. and L16:D8 without moving for 1 week. After that period, all insects were removed by sieving the beans through the sieve and the containers with bean were kept under the same experimental conditions to monitor F1 generation emerging from seeds. Based on the life cycle of untreated beans (control), the counting period of F1 was established so as to avoid an overlap of population generations: the number of F1 progeny produced was recorded daily for 30 days from the time of first adult emergence (an experiment ended 7 weeks after introducing the parental generation).

The mean values of the experiments were separated using Duncan's multiple range test (Statistica for Windows).

3. Results

The insecticidal effects of five plant extracts applied in both 30 and 100% concentrated solutions were summarized in Table 1, whereas their repellent activity and reduction of F1 progeny were summarized in Tables 2 and 3, respectively. The results showed that only *U. dioica* leaf extract (100%) and *T. officinale* belowground part extract (100%) efficiently killed adults of *A. obtectus*, and their activity increased with the time of exposure. The same solutions were highly effective as repellent agents and reduced the number of individuals in F1 progeny. However, a good level of repellency was also achieved with the same extracts in lower concentration (30%). Despite its low toxicity (Table 1), the extract from *A. millefolium* (100%), showed strong repellency (Table 2) and reduced emergence of offspring (Table 3). Other investigated extracts (*S. nigra* and *J. regia*) were ineffective in all conducted bioassays.

Table 2
Repellency of *A. obtectus* by beans treated with plant extracts

Plant extract	Percent repellency at different time intervals (mean ± S.E.)			
	4 h	12 h	24 h	48 h
Control (70 vol% eth.)	0 d	2.8 ± 0.0 d	3.9 ± 0.0 e	21.1 ± 1.6 e
U. dioica (Lf) (30%)	12.1 ± 0.4 a	18.7 ± 0.7 c	32.2 ± 1.1 c	53.5 ± 1.7 c
U. dioica (Lf) (100%)	11.6 ± 0.0 a	39.8 ± 2.1 b	79.7 ± 1.4 b	98.8 ± 2.6 a
T. officinale (Bp) (30%)	9.1 ± 1.0 b	25.4 ± 1.5 c	44.0 ± 2.2 c	66.7 ± 2.4 b
T. officinale (Bp) (100%)	10.8 ± 1.8 a	49.9 ± 2.5 a	89.5 ± 2.4 a	99.4 ± 2.5 a
A. millefolium (Fl) (30%)	9.0 ± 0.7 b	14.1 ± 0.5 c	30.4 ± 0.9 c	49.9 ± 1.2 c
A. millefolium (Fl) (100%)	10.6 ± 0.9 a	19.4 ± 0.5 c	37.7 ± 1.0 c	78.1 ± 1.7 b
S. nigra (Fl) (30%)	1.0 ± 0.0 c	2.5 ± 0.6 d	3.7 ± 0.0 e	14.4 ± 0.5 e
S. nigra (Fl) (100%)	1.2 ± 0.5 c	2.3 ± 0.6 d	4.4 ± 0.5 e	16.1 ± 0.6 e
J. regia (Lf) (30%)	1.1 ± 0.1 c	2.2 ± 0.0 d	3.7 ± 0.3 e	13.0 ± 0.5 e
J. regia (Lf) (100%)	12.0 ± 0.0 a	14.0 ± 0.4 c	16.0 ± 0.6 d	28.1 ± 1.1 d

Values are means of four observations (± S.E.). In a column, means followed by the same letter are not significantly different by Duncan's multiple range test ($P = 0.05$). Lf: extract from leaves; Bp: extract from belowground plant parts; Fl: extract from flowers.

4. Discussion

The biological activity of tested plant extracts against bacteria, fungi, viruses and pests has been reported (Sekulić et al., 1995; Bozsik, 1996; Macedo et al., 1997; Unicini Manganelli et al., 2005). As a continuum of our work on searching the alternative for control the stored product pests (Kostić et al., 1996; Sekulović et al., 1995, 1996; Živanović et al., 1996), we found that some of tested botanical agents deserve to be further investigated.

A good level of mortality, repellency and reduction of F1 progeny was achieved with extracts of *T. officinale* (100%) and *U. dioica* (100%). When these extracts were applied in 30% concentrations they did not exhibit insecticidal activity, but caused the emigration of more than 50% adult weevils in repellency bioassay (within the first 48 h of exposition, which is the period when the majority of reproduction and laying of eggs occurs, Tucić et al., 2004).

Table 3
Progeny of *A. obtectus* developed from beans treated with plant extracts (after 7 weeks of introducing the parental generation)

Plant extract	Number of offspring
Control (70 vol% eth.)	204.18 ± 1.9 a
U. dioica (Lf) (30%)	124.5 ± 1.4 b
U. dioica (Lf) (100%)	4.3 ± 0.8 e
T. officinale (Bp) (30%)	104.4 ± 2.2 b
T. officinale (Bp) (100%)	4.1 ± 1.0 e
A. millefolium (Fl) (30%)	91.1 ± 0.9 b
A. millefolium (Fl) (100%)	14.9 ± 1.1 d
S. nigra (Fl) (30%)	196.9 ± 1.8 a
S. nigra (Fl) (100%)	96.0 ± 2.6 b
J. regia (Lf) (30%)	193.8 ± 1.9 a
J. regia (Lf) (100%)	41.6 ± 2.5 c

Values are means of four observations (± S.E.). In a column, means followed by the same letter are not significantly different by Duncan's multiple range test ($P = 0.05$). Lf: extract from leaves; Bp: extract from belowground plant parts; Fl: extract from flowers.

Low contact toxicity, but good repellency and reduction of F1 progeny was achieved by application of the leaf extract of *A. millefolium* (100%). Repellent activity of some non-insecticidal agent could be attributed to the complex mixture of compounds, which are detected by the susceptible insect (Schumutterer, 1985). In case of beans treatment with leaf extract from *A. millefolium* the repellent effect was accompanied with high reduction of F1 progeny. The same as with conventional pesticides, toxicity of herbal insecticides leads to the subsequent resistance of pest. Therefore, the most appropriate mode of action of botanical control agents would be to repel the pest from the substrate and/or to affect the progeny. Such effects do not cause the pest resistance, while their effect is satisfying in the reduction of future populations.

Considering repellency and the effect of reduction of offspring as desirable modes of action against many stored products pests, we suggest the scientific rationale of using extracts from *T. officinale*, *U. dioica* and *A. millefolium* as natural control agents against bean weevil. It is not known that these volatile extracts have negative impact on human health, since they are commonly used in many pharmaceutical preparations and in human food as well. All suggested plants are widely distributed and easy grown. Furthermore, the extraction method seems to be simple and cost-effective and the application techniques could be relatively easily designed for on-farm use. We emphasize the importance of further investigation and improvement of repellent compounds of suggested plants for several reasons: (1) the primary

damage from *A. obtectus* is not from feeding, but from laying the eggs on beans; (2) insect resistance will not be provoked by toxicity; (3) in order to avoid adaptation of insect to these repellent compounds, the extracts and other commercial repellent agents should be subsequently applied; (4) it is economically rational, because the satisfying effect could be achieved with the low concentrated extracts (30%).

Acknowledgements

We thank the two referees for their valuable comments for the improvement of this paper.

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